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Inlaid Carbon Nanofiber Nanoelectrode Array as Highly Efficient Dielectrophoresis Device for Bacteria Trapping

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***Abstract*-Dielectrophoresis (DEP) is an effective microelectronic technique for trapping and manipulating biological particles in a microfluidic environment, which relies on the highly asymmetric electric field gradient created by the microelectrodes. Here we demonstrate an AC DEP technique for single-bacteria trapping using nanoelectrode arrays (NEAs) in a "points-and-lid" configuration. The NEA is based on vertically aligned carbon nanofibers (CNFs) embedded in SiO_2 matrix. The miniaturization of the electrode size provides a highly focused electric field with the gradient enhanced by several orders of magnitude. Finite element modeling indicated that the pDEP force using such NEA-based devices can be increased by over 100 times. Experiments indicated that the bacteria can be trapped instantaneously on to the exposed CNF tip with an AC voltage of ~3 V_{pp}. In most cases, a single E. coli bacterium is trapped at a single CNF site due to the screening effect. Such nano-DEP device allows the integration of millions of nanolectrodes deterministically in lab-on-a-chip devices that can be used for effective cell manipulating and concentration.**

BACKGROUND

Cell manipulation is the first critical step in single-cell analysis. Microscale DEP technique[1] has been widely used for a large number of cells because the electric field distributions can be precisely controlled by lithographically patterned electrodes in microfluidics down to single-cell resolution, i.e. at 1-50 microns. To capture smaller particles (bacteria, viruses, prions, etc.), it requires further miniaturization of electrode dimensions. One possible solution is to use one-dimensional nanostructures. It is known that the high aspect ratio nanostructures such as carbon nanotubes (CNTs) improve field emission due to the strong enhancement of electric field strength at the sharp tip[2]. Recently, Torma et al.[3] observed extremely high field gradients (~10^{23} V^2 m^{-3}) with multi-walled CNT DEP for DNA trapping. But it lacks a precise placement and control of electrode dimensions, much needed for biological studies. Here, we investigated the use of NEAs fabricated by inlaying vertically aligned CNFs (50-200 nm dia.) in SiO_2 matrix as a DEP trap in aqueous solution.

DEP is based on the polarization of particles in a non-uniform electric field.[4] The time-average DEP force on the particle in a sinusoidal field is $\langle F_{dep}(r)\rangle = \pi \varepsilon_m R^3 \mathrm{Re}[K(\omega)] \cdot \nabla|E(r)|^2$, where ε_m is the permittivity of the medium, R is the particle radius, Re[K(ω)] is the real part of complex Clausius-Mossotti factor $K=(\varepsilon_p-\varepsilon_m)/(\varepsilon_p+2\varepsilon_m)$ defined by the angular frequency (ω) dependent complex permittivity of the medium (ε_m) and particle (ε_p). For $\varepsilon_p>\varepsilon_m$, as in the case of cells and viruses, Re[K(ω)] is positive and particles move toward higher electric field, resulting in positive DEP (pDEP). The magnitude is proportional to $\nabla|E(r)|^2$, determined by the electrode geometry and voltage. For a nanoelectrode (NE), $\nabla|E(r)|^2$ can be increased over 100 times, generating a much stronger DEP force. The large pDEP force is able to counter Stokes drag force in a high-velocity laminar microfluidic flow and effectively traps E. coli at individual NEs.

MATERIALS AND METHODS

The NEAs based on inlaid vertical CNF arrays were fabricated following the similar methods reported before[5]: A microscope was used to observe the E. coli movement through an ITO electrode (as shown in Fig. 1a). The typical CNF array consists of 50-200 nm diameter and ~5 μm long fibers with an average spacing of ~400 nm. Each CNF was vertically aligned and freestanding on the surface. We controlled the condition so that only a small number of CNFs are exposed with an average spacing more than 1 micron. In a few samples (such as Fig. 1b), e-beam lithography was used to pattern the Ni catalysts into a well-separated hexagonal array of nanodots (~100 nm in dia.) which produced an array of individual CNFs. A layer of SU-8 was spin-coated and UV exposed to form the microfluidic channel and bound the NEA chip with the ITO counter electrode.

CURRENT RESULTS

The two-dimensional (2D) finite element modeling of pDEP trapping at a linear array of 12 CNF NEs at 26 V_{pp} and a flow velocity of 10 mm/s is shown in Fig. 2. Particles of 1 μm in diameter are injected at various heights simulating *E. coli*. The trajectories show that all particles injected below 12 μm height are trapped while others (including those near the 20 μm high ceiling) are deflected downwards. The flow velocity (10 mm/s) is much higher than those in micro- "points-and-lid" device (0.1-0.5 mm/s)[6] and interdigitated device (0.04-2 mm/s)[7],

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reflecting the higher trapping efficiency. The $E(r)^2$ map around a NE tip shows a maximum of ~$1.2x10^{14}$ V^2 m^{-2}, 200 times higher than that of the micro- "points-and-lid" device[6]. At 2 mm/s flow velocity, even 9 V_{pp} can trap most of the particles.

The trapping of E. coli is measured by the integrated fluorescence intensity from stained *E. coli* by focusing at a ~0.25-mm^2 area at the bottom of the channel with a dimension of 500µm(W) x 20µm(H) x 2cm(L) as shown in Fig. 3a. An *E. coli* suspension (~1×10^9/ml) is continuously passed through the microchannel. The trap is turned on and off to observe how *E. coli* are captured and released from the NEA. The DEP voltage is varied between 0 to 9 V_{pp} in a series of experiments. At a flow velocity of ~2mm/s, even 1 V_{pp} initiates evident trapping. At V_{pp} > 3 V, CNF NEs are able to generate strong DEP force to trap *E. coli* that instantaneously covered all exposed CNF tips.

Fluorescence images are taken before and after the 3 V_{pp} is turned on. When the V_{pp} is off at the beginning (Fig. 3b), *E. coli* bacteria are floating in water and generate fuzzy stretched tracks. After a 3 V_{pp} is turned on (Fig. 3c), bacteria are instantaneously snapped onto the exposed CNF tips, generating sharp bright spots while the extra bacteria are still floating. The trapped bacteria can be reversiblely released when the Vpp is turned off.

In summary, we have demonstrated the use of embedded CNF NEA in trapping small bioparticles against strong hydrodynamic drag forces in a microfluidic channel. This new nano-DEP technique can be used either as a low-density array for registration and the study of single particles or a high-density array that is tailored as an active DEP filter for sorting, separating, and concentrating bioparticles. This approach of manipulating submicron bioparticles by highly focused strong electrical fields at NEAs, promises new capabilities for cell biology research and the development of highly integrated biochips for environmental and security monitoring.

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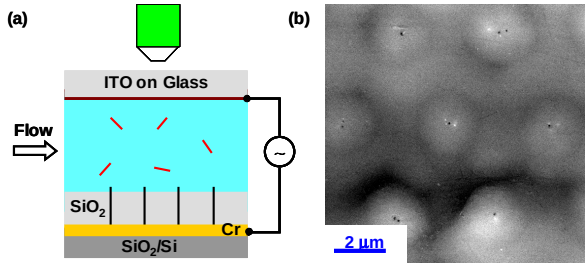

Fig. 1 (a) The layout of the "points-and-lid" DEP device with a CNF NEA at the bottom and ITO counter electrode at the top of the microchannel. The electrodes are separated by a patterned SU-8 polymer, which also forms the seal for microchannel. (b) SEM image of the top view of an inlaid regular CNF NEA that was patterned by e-beam lithography.

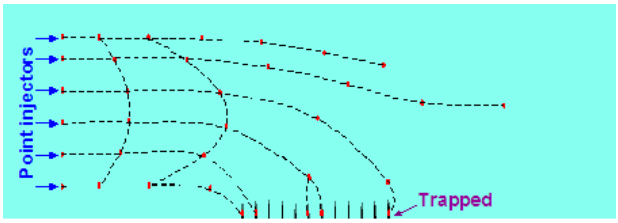

Fig. 2 Modeling of pDEP trapping on CNF NE array. The dashed lines show the trajectories of 1-µm dia. particles under the influence of Stokes drag force of a 10 mm/s flow and dielectrophoretic forces by an 10 MHz AC voltage of 26 V_{pp}.

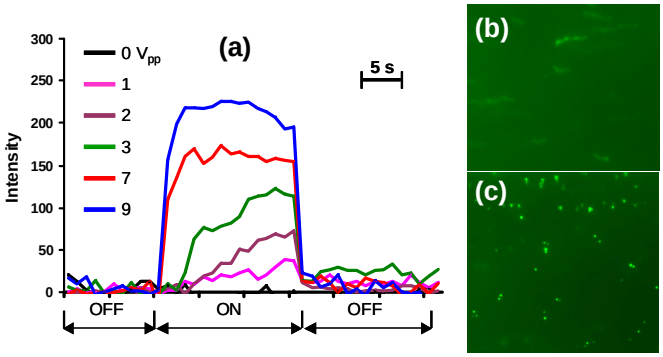

Fig. 3 (a) Integrated epifluorescence intensity when the 1 M Hz AC V_{pp} is switched on and off in a ~250 µm/s flow. (b) Epifluorescence images showing *E. coli* flowing through and trapped at CNF NEs before and after the V_{pp} is turned on.